

Phosphinate-peptide analogs for treating fibrotic diseases

The invention relates to the use of phosphinate-peptide analogs, as inhibitors of  
5 procollagen C-proteinase (PCP), for treating fibrotic diseases.

It is known that procollagen C-proteinase (PCP) is a key enzyme in fibrogenesis. It  
catalyzes the hydrolytic elimination of the procollagen propeptides from procollagens  
I, II, III and IV and also laminin V [cf. Amano S, Takahara K; Gerecke D, Nishiyama  
10 T, Lee S, Greenspan DS, Burgeson RE: Bone morphogenetic protein-1 is the  
processing enzyme for laminin 5 in human keratinocytes, Mol. Biol. Cell 7 (suppl.)  
58A (1996)]. Consequently, PCP is a key enzyme in collagen processing [cf. Olsen  
BJ: Morphogenesis: collagen it takes and bone it makes, Curr. Biol. 6: 645-647  
(1996)]. It was demonstrated in BMP-I knock-out mice that complete absence of PCP  
15 leads to incomplete collagen processing with the deposition of atypical, loose  
collagen fibrils [cf. Suzuki N, Labosky PA, Furata Y, Hargett I, Dunn R, Fogo AB,  
Takahara K, Peters DM, Greenspan DS, Hogan BL: Failure of ventral body wall  
closure in mouse embryos lacking a procollagen C-proteinase encoded by BMP-1, a  
mammalian gene related to *Drosophila tollid*, Development 122: 3587-3595  
20 (1996)].

PCP is probably also responsible for the hydrolytic elimination of the propeptide  
sequence of lysyl oxidase. The elimination of the prosequence probably leads to the  
activation of the catalytic lysyl oxidase activity of the mature form [cf. Pachenko  
25 MV, Stetler-Stevenson WG, Trubetskoy OV, Gacheru SN, Kagan HM:  
Metalloproteinase activity secreted by fibrogenic cells in the processing of prolysyl  
oxidase. Potential role of procollagen C-proteinase, J. Biol. Chem. 271: 7113-7119  
(1996)]. Active lysyl oxidase covalently links opposing collagen fibrils to each other.  
In this way, PCP also indirectly increases the biological stability of the collagen  
30 towards degradation by collagenases.

PCP, or closely related proteins, appear also to play a role in the release of TGF $\beta$ -type growth factors. Recent studies have shown that PCP-like proteases are able to liberate TGF $\beta$ -type growth factors from an inactive complex with TGF $\beta$ -binding proteins [cf. Marques G, Musacchio M, Shimell MJ, Wünnenberg-Stapleton K, Cho  
5 KWY, O'Connor MB: Production of a DPP activity gradient in the early drosophila embryo through the opposing actions of the SOG and TLD proteins, Cell 91: 417-426 (1997); Blader P, Rastegar S, Fischer N, Strähle U: Cleavage of the BMP antagonist chordin by zebrafish tolloid, Science 278: 1937-1949 (1997)]. In this case, the binding partner for the TGF $\beta$ -type growth factors is decomposed by means of  
10 specific proteolysis. Consequently, PCP may possibly also indirectly possess a TGF $\beta$ -type agonistic activity. PCP can therefore be ascribed a crucial role in fibrogenesis.

PCP activity has its origin in splicing variants of the BMP-I gene [cf. Kessler E,  
15 Takahara K, Biniaminow L, Brusel M, Greenspan DS: Bone morphogenetic protein-1: The type I procollagen C-proteinase, Science 271: 360-362 (1996)); Reddi AH: BMP-1: Resurrection as procollagen C-proteinase, Science 217: 463 (1996); Li SW,  
Sieron AL, Fertala A, Hojima Y, Arnold WV, Prockop DJ: The C-proteinase that processes procollagens to fibrillar collagens is identical to the protein previously  
20 identified as bone-morphogenetic protein-1. Proc. Natl. Acad. Sci. USA 93: 5127-5130 (1996)]. It is so far definitely known that splicing variants BMP-I-I and BMP-I-III (tld variant) are able to cut procollagen and pro-lysyl oxidase specifically. While relatively recent studies have identified further BMP-1 splicing variants, the biological function and substrate specificity of the latter have not yet been fully  
25 elucidated [cf. Janitz M, Heiser V, Böttcher U, Landt O, Lauster R: Three alternatively spliced variants of the gene coding for the human bone morphogenetic protein-1. J. Mol. Med. 76: 141-146 (1998)].

Although success has so far only been achieved in expression-cloning and purifying  
30 PCP in small yields, a large number of structural details of the enzyme are known. Thus, PCP belongs to the family of astacin proteases. The crystal structure of astacin

is known in detail. There is a very high degree of structural homology between the catalytic domain of BMP-I and astacin such that it has been possible, on the basis of this homology, to assign to many amino acids in the PCP protease domain their probable structural and biochemical function [cf. Stöcker W, Gomis-Rüth FX, Bode 5 W, Zwilling R: Implications of the three-dimensional structure of astacin for the structure and function of the astacin family of zinc-endopeptidases, Eur. J. Biochem. 214: 215-231 (1993)].

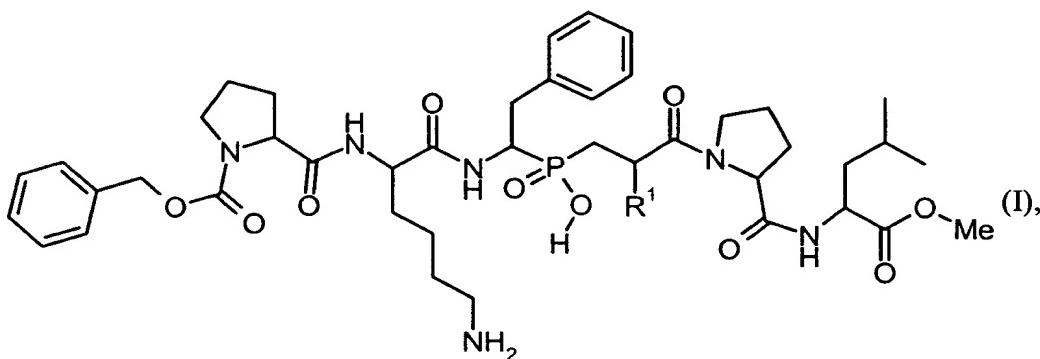
In the past, it has been possible, by means of computer-guided molecular modeling, 10 to infer the binding of substrate to the active center of the astacins in molecular detail [cf. Stöcker W, Grams F, Baumann U, Reinemer P, Gomis-Rüth FX, McKay DB, Bode W: The metzincins – Topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-endopeptidases, Prot. Sci.: 823-840 (1995)]. These studies led to the rational design 15 of phosphinate-peptide analogs which inhibit astacin with a high degree of potency. The complex between a phosphinate inhibitor and astacin has been structurally elucidated (cf. Grams F, Dive V, Yiotakis A, Yiallouros I, Vassilou S, Zwilling R, Bode W, Stöcker W: Structure of astacin with transition-state analogue inhibitor, Nature Struct. Biol. 3: 671-675 (1996)].

20 Despite the high degree of structural homology between astacin and the catalytic domain of BMP-1, it has so far been assumed that, because of biochemical differences with regard to their reaction behavior and also in their ability to be inhibited by protease inhibitors, the two proteases are in fact markedly different. For 25 example, there are biochemical differences between astacin and BMP-I with regard to their substrate specificity (as a crayfish digestive enzyme, astacin hydrolyzes collagen-like proteins relatively nonspecifically whereas PCP cuts highly specifically at only one site in the procollagen molecule and in pro-lysyl oxidase).

30 To date, only low-potency inhibitors of PCP, to which an antifibrotic effect is attributed, have been described in the literature [cf. Brenner M, Ho WB: C-proteinase

inhibitors for the treatment of disorders related to the overproduction of collagen.  
WO 97/05865].

It has now been found, surprisingly, that phosphinate-peptide analogs of the general  
5 formula (I),



in which

10

$\text{R}^1$  represents hydrogen or methyl,

and their salts and isomers inhibit PCP with a very high degree of potency and can therefore be used for the treatment and prophylaxis of fibrotic diseases.

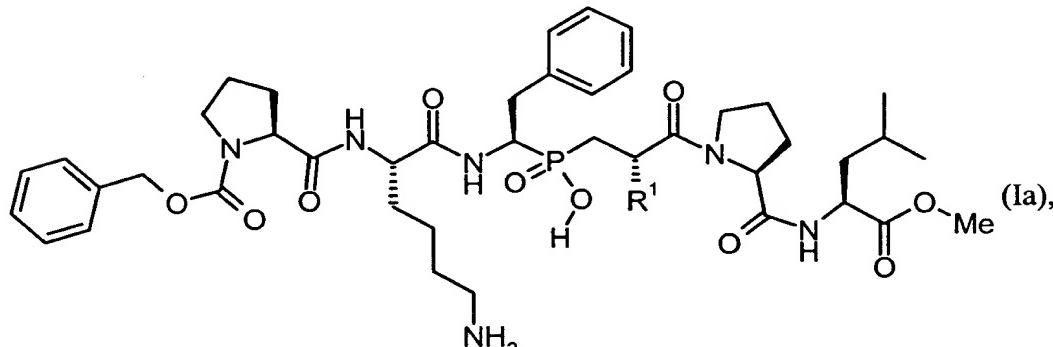
15

Within the context of the invention, preference is given to physiologically harmless salts. In general, physiologically harmless salts are salts of the compounds according to the invention with inorganic or organic acids. Preference is given to salts with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid or 20 sulfuric acid, or salts with organic carboxylic or sulfonic acids, such as acetic acid, maleic acid, fumaric acid, malic acid, citric acid, tartaric acid, lactic acid, benzoic acid, or methanesulfonic acid, ethanesulfonic acid, phenylsulfonic acid, toluene-sulfonic acid or naphthalenedisulfonic acid.

The compounds according to the invention can exist in stereoisomeric forms which either relate to each other as image and mirror image (enantiomers) or which do not relate to each other as image and mirror image (diastereomers). The invention also relates to the antipodes and to the racemic forms as well as to the diastereomeric  
5 mixtures.

The compounds of the general formula (I) can be present in all the enantiomeric and diastereomeric forms. Preference is given to those isomers in which the parts of the molecule formed from proline, lysine and leucine possess the L configuration, as well  
10 as to their salts and prodrugs.

Particular preference is given to using phosphinate-peptide analogs of the general formula (I), which have the configuration as shown,



15

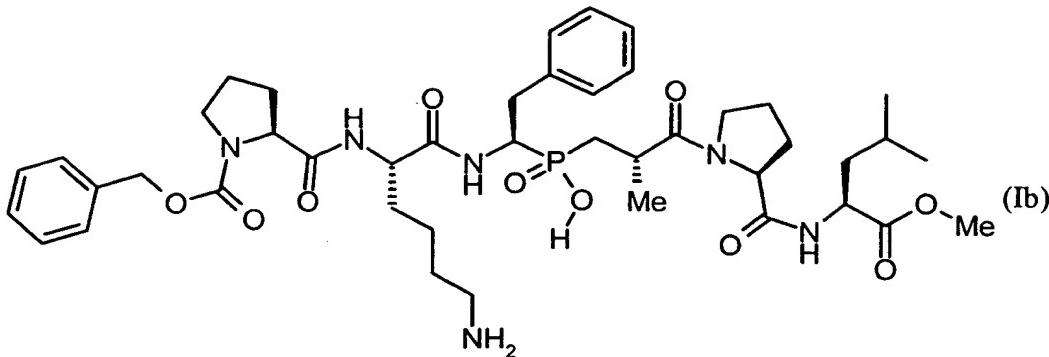
in which

R<sup>1</sup> represents hydrogen or methyl,

20

and their salts and isomers for the treatment and prophylaxis of fibrotic diseases.

Very particular preference is given to using the compound of the general formula (Ib)



designated Z--PKF(PC)APL--O-Me in that which follows,

- 5 and its enantiomers and its salts for the treatment and prophylaxis of fibrotic diseases.

The compounds of the general formula (I) are known and can in principle be prepared from corresponding phosphinate compounds using customary methods of  
10 peptide synthesis [cf., in this regard, Yiotakis A, Vassilio S, Jiracek J, Dive V: Protection of the Hydroxyphosphinyl Function of Phosphinic Dipeptides by Adamantyl. Application to the Solid-Phase Synthesis of Phosphinic Peptides, J. Org. Chem. 61: 6601-6605 (1996); Campagne JM, Coste J, Guillou L, Heitz A, Jouin P: Solid phase synthesis of phosphinic peptides, Tetrahedron Lett. 34: 4181-4184  
15 (1993)].

The results were unexpected because BMP-I, meprin and astacin differ from each other, in particular, in the so-called S1' loop. A sector from this loop is depicted here:

astacin:	T--DPYD
BMP-I:	KPPIG-Q
meprin:	I--IG-Q

This loop forms the essential part of the S1' binding pocket of the astacin-like proteases. It is here, as has been shown using the example of astacin and meprin, that the key to the differing substrate and inhibitor specificities of the two enzymes lies.

5 Meprin and astacin differ very markedly from each other in the S1' pocket. PCP additionally has two proline residues and one lysine residue in this region. As has been shown by computer modeling the protease domain of PCP, the lysine residue is very probably involved in binding the carboxyl group in the side chain of the aspartate in P1' next to the cleavage site in the procollagen. According to the model, the proline residues are in the *cis* configuration and represent a peculiarity of the

10 BMP-I subfamily among the astacins. By contrast, astacin does not cleave next to acidic residues. For these reasons, it was completely unexpected that a phosphinate which was designed for inhibiting astacin [cf. Yiallouros I, Vassiliou S, Yiotakis A, Zwilling R, Stöcker W, Dive V: Phosphinic peptides, the first potent inhibitors of astacin, behave as extremely slow-binding inhibitors, Biochem. J. 331: 375-379

15 (1998)] would prove to be an effective PCP inhibitor.

The compounds of the general formula (I) according to the invention exhibit a valuable pharmacological activity spectrum which it was not possible to foresee.

20 Surprisingly, the compounds according to the invention are notable for having a very high potency when inhibiting PCP.

The compounds according to the invention are therefore suitable for treating liver fibroses of any genesis and fibroses which are manifested in other organs.

25 These fibroses include various groups of diseases which are accompanied by a qualitative change in collagen production or by an increased deposition of collagen in the extracellular space, such as liver fibroses of differing origin, such as alcoholic liver cirrhosis, biliary cirrhosis, hepatitis of viral or other genesis, idiopathic interstitial fibroses, idiopathic lung fibroses, acute pulmonary fibroses, acute respiratory distress syndrome (ARDS), perimuscular fibroses, pericentral fibroses,

30

dermatofibromas, kidney fibroses, diabetic nephropathy, glomerulonephrites, systemic or local scleroderma, keloids, hypertrophic scar formation, joint adhesions, arthroses, myelofibroses, cicatrization of the cornea, cystic fibrosis, muscular fibroses, Duchenne's muscular dystrophy, strictures of the esophagus, Ormond's disease, Crohn's disease, ulcerative colitis and aneurysms of the large vessels.

In addition, the invention encompasses fibrotic diseases which are initiated or provoked by surgical scar revisions, plastic surgery, glaucoma, cataract fibroses, cicatrizations of the cornea, graft-versus-host disease, surgical interventions performed on tendons, nerve trapping syndromes, Dupuytren's contracture, adhesions resulting from gynecological interventions, pelvic adhesions, epidural fibroses, and diseases of the thyroid gland or the parathyroid glands, and also by metastatic bone invasion, by multiple myeloma or by restenoses.

15 Detecting PCP activity in a fluorescence dequenching test

For the purpose of detecting PCP activity, a synthetic decapeptide substrate having the sequence

20 DABCYL – Asp – Phe – Tyr – Arg – Ala – Asp – Gen – Pro – Arg – Asp (EDANS)  
– NH<sub>2</sub>

was cleaved by adding PCP. This peptide frequency corresponds to the region in procollagen  $\alpha_2(I)$  which is cleaved by PCP. The cleavage sequence is known to biochemical experts [cf. Lee ST, Kessler E, Greenspan DS: Analysis of site-directed mutations in human pro- $\alpha_2(I)$  collagen which block cleavage by the C-proteinase, J. Biol. Chem. 265: 21992-21996 (1990)], as is the dequenching test method [cf. Matayoshi ED, Wang GT, Krafft GA, Erickson J: Novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer, Science 247: 954-958 (1989)].

The concentration of the synthetic peptide was 5.6 µM, while the final buffer conditions were: 50 mM tris, pH 7.5, 150 mM NaCl and 0.005% Brij35.

The kinetic test was carried out in the following manner: the fluorescence substrate  
5 was dissolved in 90 µl of reaction buffer. The kinetics of the conversion of the  
substrate by PCP were detected, in duplicate determinations, by measuring the  
fluorescence (ex. 355 nm/em. 538 nm) at from 0 to 120 min. The reaction was started  
by adding a suitable activity of PCP dissolved in 10 µl of reaction buffer. The  
emission, as a measure of the proteolytic activity, was measured at time intervals of  
10 initially 5 min and then subsequently at longer time intervals. The spontaneous  
decomposition of the fluorescence substrate in the added presence of reaction buffer  
lacking PCP activity was measured as the negative control. The fluorescence  
measurements took place at 37°C over a period of approx. 4 h. After the reaction had  
come to an end, the substrate was completely decomposed by adding proteinase K  
15 (Boehringer Mannheim, 1.44 µg per reaction mixture, dissolved in 10 µl of PBS) and  
incubating at 37°C for 20 min. The fact that the hydrolysis was complete was shown  
by there being no further increase in the fluorescence with time.

The relative conversion of substrate in % of the total quantity was calculated from:

20

$$\% \text{ conversion} = (F_t - F_{-t}) \div (F_{\text{total}} - F_{\text{ini}}) \times 100\%$$

where  $F_t$  is the relative fluorescence after a time interval  $t$  while incubating with  
PCP.  $F_{-t}$  is the corresponding relative fluorescence after the time interval  $t$  without  
25 PCP being added,  $F_{\text{total}}$  is the fluorescence following total hydrolysis produced by  
adding proteinase K, and  $F_{\text{ini}}$  is the initial relative fluorescence before starting the  
reaction by adding proteinase K.

In the test, the PCP activity used was typically adjusted such that approx. 20% of the  
30 substrate was converted by inhibited enzyme within the measurement period.

- 10 -

Figure 1 shows typical reaction kinetics (% conversion) which were obtained when adding the phosphinate inhibitor Z---PKF(PC)APL---O-Me.

The percentage inhibition obtained in the added presence of the inhibitors was  
5 calculated as follows:

$$\% \text{ inhibition} = 100\% \times \% \text{ conversion (with inhibitor)} \div \% \text{ conversion (without inhibitor)}$$

10 Figure 2 depicts the concentration-effect relationship of a phosphinate inhibitor.

Determining the specificity of PCP inhibitors by carrying out in-vitro investigations using the astacin-like protease meprin

15 Meprin is an enzyme which belongs to the astacin protease family and which is found in humans (Stöcker W, Gomis-Rüth FX, Bode W, Zwilling R: Implications of the three-dimensional structure of astacin for the structure and function of the astacin family of zinc-endopeptidases, Eur. J. Biochem. 214: 215-231 (1993)). The meprin activity test was carried out in a completely analogous manner to the in-vitro assay  
20 for measuring PCP activity. Instead of the recombinant PCP, use was made of human meprin, which also transforms the fluorescence-labeled decapeptide.

Figure 3 shows the specificity of the highly active phosphinate-peptide analog Z---PKF(PC)APL---O-Me for PCP. Even at concentrations of up to 100 nM, meprin is  
25 only slightly inhibited (in a dose-dependent manner) in its transformation of the fluorescence-labeled peptide.

Demonstration of biological activity

30 The biological activity of the substances can be demonstrated in cell culture assays and in vivo. For example, after administering the inhibitors, it is possible, in human

cell lines, to measure the decline in the concentration of free procollagen  $\alpha_1$ (III) propeptide in the supernatants, because this peptide is released by PCP activity. The PIICP concentrations in the supernatant can be measured using a recently established assay [cf. Burchardt ER, Schröder W, Heke M, Kohlmeyer J, Neumann R, Kroll W:

- 5 Expression cloning of C-terminal procollagen (III) propeptide and its use in a novel serum assay to monitor liver fibrogenesis, Hepatology 26: 487A (1997)].

In order to demonstrate the antifibrotic effect of the substances in the liver, it is possible, for example, to use the animal model of acute [cf. Johnson SJ, Hines JE,

- 10 Burt AD: Phenotypic modulation of perisinusoidal cells following acute liver injury: a quantitative analysis. Int. J. Exp. Path. 1992; 73: 765-772 (1992)] or chronic [cf. McLean E, McLean A, Sutton P: Instant Cirrhosis. An improved method for producing cirrhosis of the liver in rats by simultaneous administration of carbon tetrachloride and phenobarbitone, Br. J. Exp. Pathol. 1969; 50: 502-506 (1969)]  
15 carbon tetrachloride-induced liver damage, the model of liver fibrosis due to bile duct ligation [cf. Kountouras J, Billing B, Scheuer P: Prolonged bile obstruction: a new experimental model for cirrhosis in the rat, Br. J. Exp. Pathol. 1984; 65: 305-311 (1984)] or the liver fibrosis which is induced by heterologous serum [cf. Bhunchet E, Wake K.: Suppression of experimental hepatic fibrosis by administration of vitamin  
20 A, Lab. Invest. 1985; 52: 182-194 (1984)]. It is also possible to use other animal models in which liver fibrosis occurs for demonstrating the antifibrotic effect.

- Depending on the organ in which the fibrosis is manifested, or on the nature of the fibrotic damage, it is also possible to use animal models for other manifestations of  
25 fibrosis, for example in the heart, in the kidneys, in the lungs, in the skin or in other organs.

- The reduction in collagen deposition can be demonstrated, for example, by determining the content of hydroxyproline [cf. Gerling B, Becker M, Waldschmidt J,  
30 Rehmann M, Schuppan D.: Elevated serum aminoterminal procollagen type-III-peptide parallels collagen accumulation in rats with secondary biliary fibrosis,

Hepatology 1996; 25: 79-84 (1996)] in the fibrotic organs or by means of quantitative morphometry [cf. Kauschke SG, Knorr A, Olzen M, Burchardt ER: Expression of collagen (III) as determined by quantitative PCR and its correlation with extracellular collagen deposition in the rat CCl<sub>4</sub> model of liver fibrosis. Hepatology 26: 538A 5 (1997)].

Figure 1 shows the transformation of the DABCYL-EDANS-decapeptide substrate by recombinant PCP as a function of time. The enzyme activity is almost completely inhibited in the presence of 100 nM inhibitor.

10

Figure 2 shows the concentration-effect relationship of the phosphinate inhibitor Z---PKF(PC)APL---O-Me.

15

Figure 3 shows the specificity of the highly active phosphinate-peptide analog Z---PKF(PC)APL---O-Me for PCP. Even at concentrations of up to 100 nM, meprin is only slightly inhibited, in a dose-dependent manner, in its transformation of the fluorescence-labeled peptide.

20

The present invention also includes pharmaceutical preparations which, in addition to inert, nontoxic, pharmaceutically suitable adjuvants and excipients, also comprise one or more compounds of the general formula (I), or which consist of one or more active compounds of the formulae (I), and also processes for producing these preparations.

25

In these preparations, the active compounds of the formulae (I) should be present at a concentration of from 0.1 to 99.5% by weight, preferably of from 0.5 to 95% by weight, of the total mixture.

30

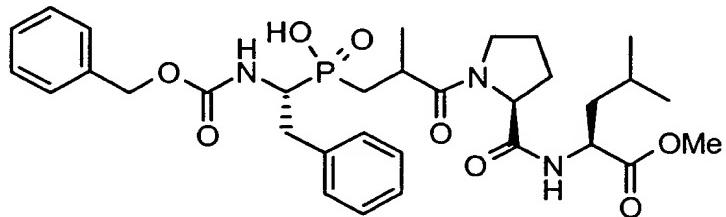
In addition to the active compounds of the formulae (I), the pharmaceutical preparations can also comprise other pharmaceutical active compounds.

The abovementioned pharmaceutical preparations can be produced in a customary manner using known methods, for example using the adjuvant(s) or excipient(s).

- In general, it has proved to be advantageous, in order to achieve the desired result,
- 5 administer the active compound(s) of the formulae (I) in total quantities of from about 0.01 to about 100 mg/kg, preferably in total quantities of from about 1 mg/kg to 50 mg/kg of body weight per 24 hours, where appropriate in the form of several individual doses.
- 10 However, it can, where appropriate, be advantageous to deviate from the said quantities, depending on the nature and body weight of the individual being treated, on the individual response to the drug, on the nature and severity of the disease, on the nature of the preparation and its administration, and on the time or time interval at which the administration is effected.
- 15 The preparation of the compounds of the formula (I) according to the invention is demonstrated below with the aid, by way of example, of an exemplary synthesis of the compound of the formula (I, R = methyl), to which the present invention is not, however, limited. Unless otherwise stated, all the quantity data given below refer to
- 20 percentages by weight.

Ex. 1a: (1*R*)-1-{[(Benzylxy)carbonyl]amino}-2-phenylethyl{3-[(2*S*)-2-  
({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-  
1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid

25

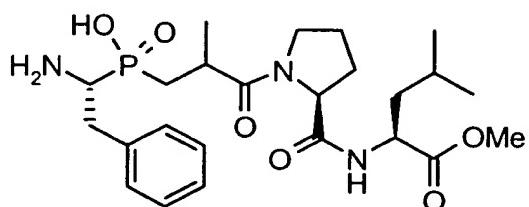


4.0 eq. of ethyldiisopropylamine, 1.50 eq. of benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBop) (J. Martinez et al., J. Med. Chem. 1988, 28, 1874; J. Costre, D. Le-Nguyen, B. Castro, Tetrahedron Lett., 1990, 31, 2055) and, after from 2 to 5 min, 1.0 eq. of Pro-Leu-OMe-trifluoroacetic acid salt (prepared 5 using standard methods of peptide chemistry, cf., e.g., Houben-Weyl, 4th edtn.: Methoden der Organischen Chemie (Methods in organic chemistry), Volume XVI/-2, Synthese von Peptiden Teil 1 und Teil 2 (Synthesis of peptides, Part 1 and Part 2), Georg Thieme Verlag, Stuttgart 1974) are added, one after the other, at 0°C and under argon, to a solution of from 1.4 to 1.5 eq. of [(1- 10 benzyloxycarbonyl)amino]-2-phenylethyl)-(2-carboxy-1-propyl)hydroxypyrophosphonic acid (prepared in analogy with WO 89/10961, p. 72, Ex. [14]) in absolute dichloromethane (from approx. 0.1 to 0.15 mol/l). After 15-30 min, the ice cooling is removed and the mixture is stirred overnight at room temperature. It is then diluted with dichloromethane and washed consecutively with saturated sodium hydrogen 15 carbonate solution, 1N hydrochloric acid solution and saturated sodium chloride solution, dried over magnesium sulfate and concentrated in vacuo.

Yield: 133.7 mg of a yellowish oil as the crude product (no yield given since the product contains solvent residues)

20 LC-MS: rt (%), m/z (%) = 4.066 (34.6%), 630 (100, M+H); 4.197 (52.7), 630 (100, M+H).

Ex. 1b: (1*R*)-1-Amino-2-phenylethyl{3-[*(2S*)-2-((*(1S*)-1-(methoxycarbonyl)- 25 3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid



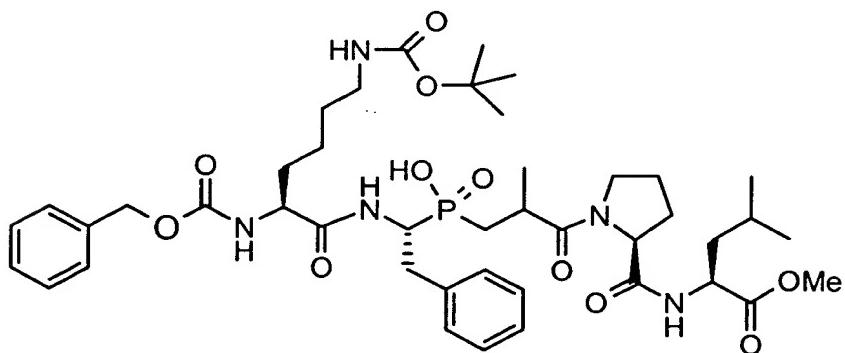
The protected amine derivative from Ex. 1a is dissolved in ethanol (approx. 0.05 mol/l), after which a catalyst (10% palladium on charcoal) is added to the solution under argon. The suspension is stirred vigorously at room temperature and 5 under an H<sub>2</sub> atmosphere (normal pressure) for approx. 2 hours before being filtered through celite (subsequently washed thoroughly with ethanol). The filtrate is concentrated in vacuo and dried under high vacuum.

Yield: 80.4 mg (97.3% of theory)

LC-MS: rt, m/z (%) = 2.706, 496 (100, M+H)

10 HPLC: rt (%) = 5.80 (32.5), 5.91 (51.5).

Ex. 1c: (1*R*)-1-((2*S*)-2-[(Benzylcarbamoyl)amino]-6-[(*tert*-butoxy-carbonyl)amino]hexanoyl)amino)-2-phenylethyl{3-[(2*S*)-2-((1*S*)-1-(methoxycarbonyl)-3-methylbutyl)amino]carbonyl}tetrahydro-1*H*-pyrrol-15 1-yl]-2-methyl-3-oxopropyl}phosphinic acid



4.0 eq. of ethyldiisopropylamine, 1.50 eq. of PyBop and, after from 2 to 5 min,  
20 1.45 eq. of Z-(NHBoc)-Lys-OH are added, one after the other, at 0°C and under argon, to a solution of 1.0 eq. of the compound from Ex. 1b. After 15-30 min, the ice cooling is removed and the mixture is stirred overnight at room temperature. It is diluted with dichloromethane and consecutively washed with saturated sodium hydrocarbonate solution, 1N hydrochloric acid solution and saturated sodium chloride solution, dried over magnesium sulfate and concentrated in vacuo.

- 16 -

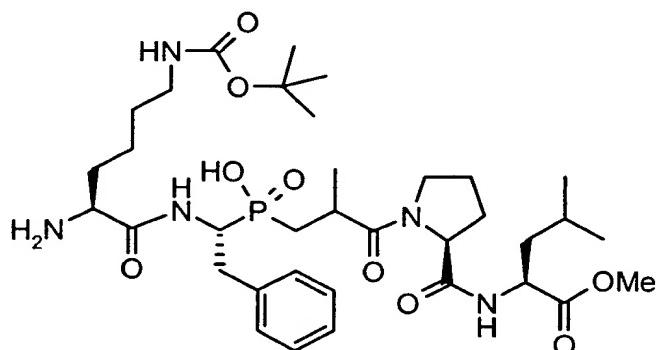
Crude yield: 207 mg of a viscous oil, LC-MS: rt (%), m/z (%) = 4.456 (11.2%), 858 (100, M+H); 4.549 (32.7), 858 (100, M+H). The crude product is purified by means of preparative RP-HPLC.

Yield: 40 mg (32.8% of theory).

5

Ex. 1d: (1*R*)-1-((2*S*)-2-Amino-6-[*tert*-butoxycarbonyl]amino]hexanoyl)-amino)-2-phenylethyl {3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid

10



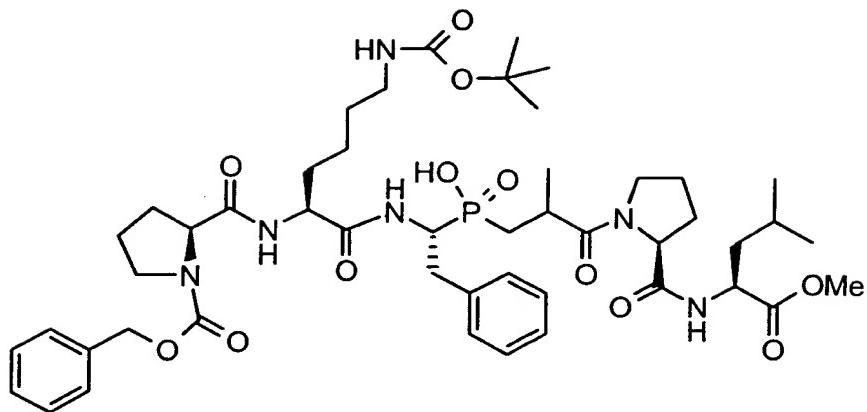
The protected amine derivative from Ex. 1c is dissolved in ethanol (approx. 0.05 mol/l), after which a catalyst (10% palladium on charcoal) is added to the 15 solution under argon. The suspension is stirred vigorously at room temperature and under an H<sub>2</sub> atmosphere (normal pressure) for approx. 2 hours before being filtered through celite (subsequently washed thoroughly with ethanol). The filtrate is concentrated in vacuo and dried under high vacuum.

Yield: 31 mg (85.4% of theory)

20 LC-MS: rt (%), m/z (%) = 2.97 (85.8%), 724 (100, M+H).

Ex. 1e: (1*R*)-1-((2*S*)-2-Amino-6-[*tert*-butoxycarbonyl]amino]hexanoyl)-amino)-2-phenylethyl {3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid

25



4.0 eq. of ethyldiisopropylamine, 1.50 eq. of PyBop and, after from 2 to 5 min,  
5 1.45 eq. of Z-Pro-OH are added, one after the other, at 0°C and under argon, to a  
solution of 1.0 eq. of the compound from Ex. 1d. After 15-30 min, the ice cooling is  
removed and the mixture is stirred overnight at room temperature. It is diluted with  
dichloromethane and washed consecutively with saturated sodium hydrocarbonate  
solution, 1N hydrochloric acid solution and saturated sodium chloride solution, dried  
10 over magnesium sulfate and concentrated in vacuo. 50 mg of crude product are  
obtained.

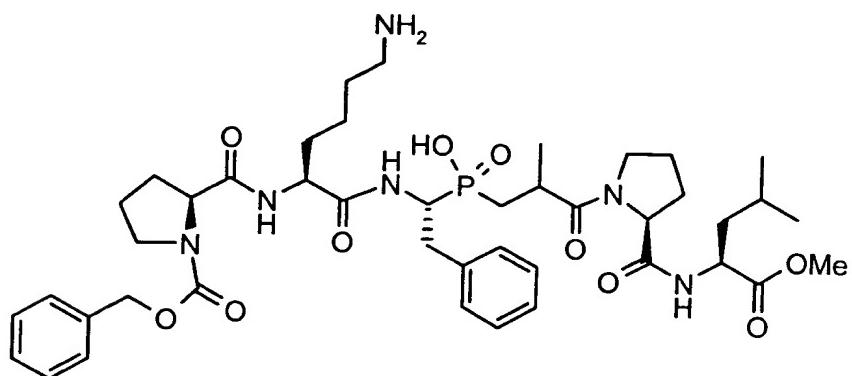
LC-MS:  $r_t$  (%), m/z (%) = 4.394 (25.9%), 955 (100, M+H); 4.509 (44.6), 955 (100,  
M+H). The crude product is purified by means of preparative RP-HPLC.

Yield: 24.5 mg (59.9% of theory).

15

Ex. 1f: Preparation of the compound of the formula (I), R = methyl

(1*R*)-1-((2*S*)-6-amino-2-[(*(2S*)-1-[(benzyloxy)carbonyl]tetrahydro-1*H*-pyrrol-2-  
yl]carbonyl)amino]hexanoyl)amino)-2-phenylethyl{3-[(2*S*)-2-((1*S*)-1-(methoxy-  
20 carbonyl)-3-methylbutyl)amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-  
oxopropyl}phosphinic acid



0.3 ml of trifluoroacetic acid is added dropwise to 24.50 mg (0.03 mmol) of the compound from Ex. 1e in an ice-cooled mixture consisting of 0.3 ml of methylene

- 5 chloride and 0.03 ml of water. The cooling is removed and the mixture is stirred at room temperature for two hours before being concentrated in vacuo and the residue being thoroughly dried under high vacuum.

**Yield:** 21.0 mg of a colorless solid (95.8% of theory)

LC-MS: rt, m/z (%) = 3.057 (both stereoisomers), 855 (100, M+H)

- 10 RP-HPLC rt (%) = 2.18 (48.7), 2.42 (46.0).